On a Remarkable New Type of Protistan Parasite.

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With Plates 29 and 30 and 2 Text-figures.

In the course of a study of the flagellates, which develope in simple cultures of goat's dung, we were afforded recently the opportunity, by the kindness of Drs. E. H. Ross and J. W. Cropper, of examining the contents of the rumen and other parts of the digestive tract of a goat, with a view to ascertaining what flagellates occurred in the active condition in the goat. The results of our observations in this connection will be dealt with in another memoir. The object of this communication is to describe certain remarkable parasites which we found, which appear to be of a nature quite distinct from any other protist of which we are aware. These parasites occur under two characteristic forms, which we have distinguished respectively as crescents and ovals. We are strongly inclined to regard these two principal types as being different phases of one parasite. We have examined, up to the present, six goats, and in the rumen of each, either the crescents, or the ovals, or both forms have occurred in enormous numbers—far exceeding those of the ciliates or ordinary flagellates present. A comparison of the occurrence of the two forms is given in the following table:

Goat.	Crescents.	Ovals.
No. 1 . . .	Relatively infrequent	Abundant.
No. 2 . . .	Very abundant	Very abundant.
No. 3 . . .	Very abundant	Doubtful if present.
No. 4 (first time of examination)	Very scanty	Very abundant.
No. 4 (second time).	Much more frequent	Much less numerous.
No. 4 (third time) .	Numerous	Abundant.
No. 5 (first time) .	Present (see Note)	Present (see Note).
No. 5 (second time).	Fairly numerous	Numerous.
No. 6 . . .	Abundant	Very abundant.

Note.—The first three goats and the last one were killed, but in the case of the remaining two some of the fluid contents of the rumen were obtained by means of a stomach-tube. In the first examination of No. 5, only an extremely small quantity of material was obtained—scarcely any in fact—and this observation afforded no precise indication of the numbers of the parasite present.

Our attention was first directed particularly to the crescents because of their vigorous movements, and the fact that, in individuals which were moving but little or else were at rest, a single, conspicuous flagellum could be seen to be attached to the concave side of the parasite.[1] On account of this characteristic appearance we have given to this form the new generic name, Selenomastix. Before describing the parasite, however, we should point out that the crescents, at all events, have been undoubtedly observed before, for in the existing literature we are aware of two references which relate to this organism; but neither of them furnishes any true indication of its peculiar characters. The first record occurs in a short note by Certes ('Bull. Soc. Zool. France,' vol. xiv, 1889, p. 70), on the micro-organisms in the rumen of ruminants. This author observed, associated with the ciliates, a flagellate in the form of a crescent, which assumed at times an S-shape, and had its flagellum inserted at the middle

[1] We may add that the flagellum was actually observed first by Dr. E. H. Ross, in a preparation which he stained rapidly by the jelly-method, on being made acquainted with our discovery of the parasite.

of the incurved part of the body. The size is given as 8–9 μ long by 2–3 μ wide. Certes proposed the name Ancyromonas ruminantium for the parasite, although he recognised that there was very considerable difference between the new organism and the known species of Ancyromonas. (As will be seen from the subsequent account, the new parasite has nothing whatever to do with Ancyromonas.) Certes goes on to say that he found also in the rumen Sarcinæ, the predominating forms being ovoid, hyaline and small, about 8–10 μ by 2–3 μ. The smallest forms showed sometimes the commencement of budding,. and in consequence might be associated with yeasts. Others, the great majority in fact, multiplied by fission. The ovals which we have found have certainly nothing to do either with yeasts or Sarcinæ. It seems very probable, however, that they are the same thing as the predominating ovoids of Certes, which he erroneously connected with the smaller, budding organisms; these latter may have been of the nature of Sarcinæ. The second reference is a brief note by Kerandel, in a paper on hæmatozoa observed in the Congo ('Bull. Soc. Path. exot.,' vol. ii, 1909, p. 208), to the effect that he had observed from the œsophagus of an antelope (Cephalophus sp.) bodies similar to those previously noted by Certes. He refers to the conspicuous cilia inserted in the middle of the concave side of the body (cf. below, p. 440). Neither author gave any figures of the parasites, but it is apparent that both were dealing with the same creature which is here described. Hence, the new parasite must bear the name Selenomastix ruminantium (Certes).

The rumen is undoubtedly the principal habitat of Selenomastix. A small drop of the fluid contents taken from any part of this bulky organ (in the case of killed goats) has always been full of one or other form of the parasite. The crescents have been found also, in sparing numbers, in the rumen of a sheep. Hence this creature appears to be a common parasite of ruminants, and, at any rate, in the goat is very abundant. The parasites also occur in small numbers

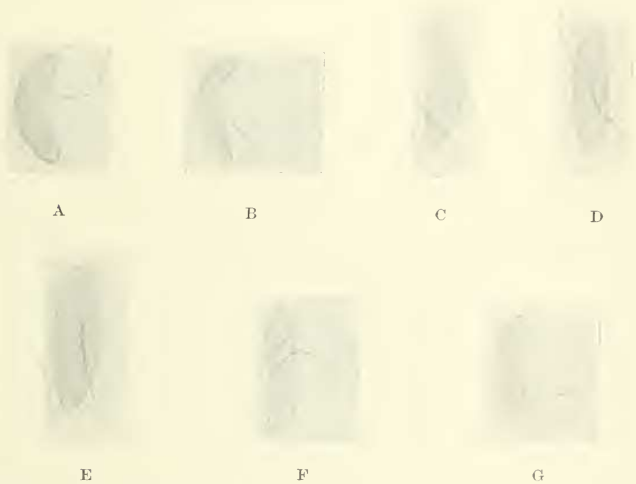
in the reticulum, and a few have been seen in the true stomach, but they have never been observed in the intestine, cæcum or rectum. In the last-named region of the alimentary canal, cysts and spores of one kind and another occur, the determination of which is a very puzzling matter, and it is quite possible that some one of these represents a resistant phase of the parasites; but we have not succeeded in recognising anything corresponding to the characteristic ovals. Certainly no crescents are to be found, nor have they developed in any of the many cultures which we have made from the fæces, both of goats which we know to have been infected and of others which in all likelihood also were. In this feature Selenomastix agrees with the Ciliates and the ordinary Flagellates which occur in an active phase in the rumen. Like these, further, this new parasite appears to be purely an inhabitant of the fluid contents of the rumen. We have carefully examined the wall, both externally and internally, for any indication of cysts, large or small, but none have been visible. (None of the goats examined have shown any signs of the "cysts of Gilruth.")

We will first describe the appearance, behaviour and structure of the crescents, and can then readily compare the corresponding features in the ovals. Seen living and freshly removed from the rumen, either with or without the addition of a small drop of water or normal saline, all the crescents have a very similar and characteristic appearance, but show a considerable range of variation in size. The body has a uniform, homogeneous, dull-looking appearance. We have never observed the crescents alter in shape at all; they are certainly not amœboid or "metabolic," and we have never seen them assume an S-shaped form, as mentioned by Certes (cf. below, p. 439). Many, if not most, of the crescents possess a definite envelope, as is seen from stained preparations, but it does not stand out at all in life (contrast the ovals, below). No granules of any kind, or vacuoles, are noticeable in the protoplasm.

If examined as soon as possible after removal from the

rumen, a large proportion of the crescents are usually actively motile. As already mentioned, Selenomastix possesses a single,[1] relatively large flagellum, which appears to be inserted in the body, as a rule, about the middle of the concave side (Text-figs. A–E). The movements are very varied. We have distinguished the following kinds, but

TEXT-FIGS. A—G.



Crescents drawn either living or after fixation with osmic acid vapour. A–C seen from the side; D and E showing the origin of flagellum from concave face; F and G two different stages in division.

though we have studied them very closely we are in some cases not quite certain how they are caused. (1) The flagellum is directed behind and undoubtedly acts as a pulsellum, causing forward progression of the body, in a rather irregular, zig-zag, but not spiral manner. We have not observed a definite reversal of the direction of this movement, i. e. in the opposite sense, the other end of the creature suddenly leading the way. This appears to be the

[1] Many of the crescents have two flagella, but these are individuals undergoing division (Text-figs. F and G).

least common type of movement, but we regard it as very important, since it provides a distinct indication of antero-posterior polarity. (2) The body oscillates to and fro, or turns round completely, one and a half times, or even twice, in the plane of its long axis, i. e. turning a somersault as it were; no forward progression is effected. This movement may be quite rapid. When it can be seen, the flagellum stands out well from the body; it may cause this movement, but we feel undecided, for we have noticed that when a crescent is becoming a little sluggish and the oscillations take place at longer intervals and not so rapidly, a premonitory tremble of the body occurs the instant before the movement. (3) Some parasites, which are moving only at intervals, can be seen to turn slowly from one side to another, this time around the long axis. In these cases the flagellum appears to be quite passive, and to turn over after the body. We certainly consider this movement is caused by the body and not by the flagellum. Moreover, now and again a crescent can be seen oscillating more or less, when it is impossible to see a flagellum in connection with it, and though of course it is sometimes difficult to make out the flagellum, nevertheless, with the aid of good lenses and critical illumination, this can usually be clearly seen; further, crescents without a flagellum do undoubtedly occur. Hence we feel persuaded that movements caused by the body alone and not by the flagellum do take place (cf. also the ovals).

The movements of most of the crescents cease very soon after being removed from the rumen—surprisingly soon in some cases. After about an hour few are still to be found active, and their movements have become irregular and spasmodic. Even in a quantity of the fluid contents kept in a warm water-bath, at from 30°–35° C., in three or four hours the parasites were all motionless. Crescents may have short resting intervals and may then become actively motile again; but when all or nearly all the crescents in a particular area of the drop under examination remain motionless, they may be regarded, we think, as being dead. In the rumen-contents

just referred to, though they were frequently examined during two days, we never found any active parasites after about four hours. The third goat examined furnished a remarkable illustration of this point. It was killed, and quantities of the rumen contents, taken from different parts, were put into two small dishes, which had been previously warmed. Not more than a quarter of an hour elapsed before these were examined, but although the crescents were extremely abundant, not one of them was active, though the flagellum could be made out in many. A warmed pipette was then taken and a fresh quantity of the contents obtained and immediately looked at; in the cover-slip preparations made here and there were areas of active parasites, though in other places they were motionless.

We consider that, in general, it is the lowering of the temperature which renders the parasites motionless, though we have come across exceptions. Thus, on one occasion, in a cover-slip preparation which had been kept at about 30° C. for twenty-four hours, a few parasites were still feebly motile. Again, in an endeavour to cultivate the parasites on agar plates, we have found two or three individuals still motile five days after being removed from the goat. In this character of extreme susceptibility to change of environment, Selenomastix agrees with the peculiar Ciliates and the Flagellates also present in the rumen; the Heterotrichous forms (Entodinium, Ophryoscolex) are even more quickly rendered motionless—indeed, frequently one can no longer find an individual still active—while the Flagellates are apparently just about as susceptible as Selenomastix, remaining active for two or three hours. On the other hand, the motile bacteria which occur (bacilli, spirillar forms) remain active for a much longer time.

We may add here that no development of the parasites occurs when "cultivated" outside the body, so far as we have been able to ascertain. We have tried simple agar plates, varying the strength and consistency of the medium. There is no further multiplication or apparent increase in number

either of the crescents or of the ovals. Ovals can be recognised longer than the crescents and are probably more resistant (cf. below); but by the end of eight days the medium is so overrun by bacterial and fungal growths that nothing else can be made out. Dr. Ledingham, of the bacteriological department here, kindly had cultures of the parasites made for us on different media, and he also informed us that no development took place.

Structure.—Selenomastix ruminantium presents some highly remarkable features in its morphology and minute structure. The usual and typical form is slightly crescentic (figs. 1, 5, 7, 18); in the larger individuals it is often very like a banana (figs. 25, 26). The parasite is never sickle or S-shaped; and this is true however big and long the crescents may be. Further, in the vast majority of parasites, if not in all, the curve of the crescent lies in one plane, *i. e.* it is not spiral like that of a spirillum. Viewed in this plane the parasites have the appearance of considerably elongated ovals (figs. 8, 9). Nevertheless, now and again, but very rarely, one gets the impression of the slightest possible twist in the axis of the parasite; thus in fig. 21 and to a less extent in Text-fig. E, there is an indication of one end of the body pointing rather in the opposite direction to the other. We have looked particularly for indications of a spiral character of the organism in life, and this appearance, which we have observed in only very few individuals, is the only one we have obtained. Moreover, we do not feel at all certain that this appearance corresponds to a permanent twist, however slight, in the body. For we have noticed, in watching certain individuals progressing forwards—sometimes, too, individuals in which no flagellum could be made out—that the hinder part of the body moves slightly to and fro, laterally, in a zig-zag manner, distinctly more so than does the front part, which is kept fairly steady; this may perhaps be caused by a slight voluntary twisting of the hinder end of the body, first in one sense and then in the opposite one, this movement serving to propel the body (cf.

Certes' remarks, above, though there is never anything approaching an S-shape).

The concavity of the crescent may be only very slight (figs. 1, 14, 16), or may be practically absent (figs. 2, 4, 11, 12); small forms often appear thus. Individuals immediately resulting from division may be pyriform, differing from the ovals in having one end broader than the other (figs. 34, 35). We have not succeeded in finding an individual with both sides markedly convex, which at the same time possesses a flagellum; in other words, we have not found a typical oval with a flagellum. The nearest approach to an oval shape is seen in figs. 4 and 11, and these individuals, though they still come in the category of crescents (for one side is practically straight), nevertheless closely resemble certain ovals. When seen more or less in the plane of the curve, the body of a crescent can be distinguished from that of an oval by the fact that its ends are narrower and more tapering (figs. 8, 9).

The flagellum is apparently always attached to the concave (or straight) side of the body, and in the majority of cases its point of insertion is about the middle of this side. But this point varies to a certain extent, particularly in the smaller forms, where the flagellum may arise much nearer to one end (figs. 1, 3, also 18); we have never found it, however, actually terminal in origin. It is possible that this variation in the point of attachment of the flagellum may be partly dependent upon the process of division. The question of the orientation of the body is one of much difficulty. If the middle point of insertion of the flagellum represents approximately the anterior end, then it is obvious that the body is greatly extended laterally. It is certain, however, that the parasite never progresses forwards in a direction at right angles to its long axis, i. e. broadside on, as it were. We have, in fact, no reason to suppose that this is the right view to take. The only clue to an orientation of the body is the indication we get from certain movements of progression of the parasites of an antero-posterior polarity; in such cases

the end nearest to which the flagellum is inserted goes first and may be regarded as anterior, the flagellum itself being directed backwards.

As regards the dimensions of the parasites, crescents of average medium size have a length of 9·5 to 11 μ and a breadth of 2 to 3 μ (Text-figs. A–D and figs. 1–8, 11–16), the length of the flagellum being about 8–9·5 μ. The largest (single) individual we have observed (on a "wet-fixed" film) is 12·5 μ long by 3·25 μ broad and the flagellum is 15 μ long (fig. 10); on a "dry," Giesma smear, the largest crescent found measures 13·5 μ in length by 3·75 μ in width,[1] the flagellum being 12 μ long (fig. 77). The smallest crescent observed, just in the act of separating after division (fig. 33), is only 4·25 μ by 1·9 μ; another small one (fig. 35) is 6·25 μ long and rather stouter, being 2·5 μ broad. Between these extremes all intermediate sizes occur.

The flagellum itself may be as long as 16 μ (fig. 2) or as short as 7·5 μ (fig. 12); its length does not bear any very close relation to the size of the parasite, the small individual of fig. 5 having a very long flagellum, while the large parasite of fig. 19 has a relatively short one. A remarkable fact bearing upon the structure of the flagellum is brought out by "dry" Giemsa-stained smears. In wet-fixed films the flagellum does not apparently differ much from that of an ordinary flagellate; it has, perhaps, a thicker and stronger appearance on the whole, though it usually thins out a little and becomes more tapering towards the free end. On Giemsa smears, however, the flagellum is frequently seen to be more or less broken up into separate bands or fibrils, often throughout the greater part of its length (figs. 75–77); or else it has split into two or three fibrils near the free end. This appearance has certainly nothing to do with division, which is quite different (cf. below); moreover, we have never seen it in

[1] Some of the parasites on Giemsa smears are possibly a little too wide relatively, having been flattened out slightly in making the preparation; on the other hand, the parasites on wet-fixed films are probably slightly (uniformly) contracted.

wet-fixed films. Apparently the somewhat rougher treatment of the parasites in making a Giemsa smear—perhaps the drying—may cause the flagellum, in certain cases, to be partially broken up into component fibrils. This observation is very interesting, because it points to the flagellum having a structure rather different from that of most ordinary flagellates, for, in the course of our work on the forms which crop up in the fæcal cultures (e. g. Monas, Cercomonas, Bodo), we have never observed such a splitting of the flagellum, and we have made numerous Giemsa smears.[1]

It is apparent from wet-fixed preparations that a definite membrane or envelope surrounds many, if not all, the crescents. It is curious that, within a short distance of one another, parasites can be found, both single individuals and forms undergoing division, which show indications of this envelope to a very varying extent. Thus it may be seen, standing off from the general protoplasm of the body, only at one end (figs. 23, 35, 36); or at both ends (figs. 6, 16); or along one side (fig. 2); or nearly all round the body (figs. 3, 19, 27). In others, again, it is not discernible at all (cf. figs. 1, 4, 7). We are uncertain whether these different appearances represent the actual condition in life, or whether they are to some extent due to the body-protoplasm having undergone a certain amount of shrinkage away from the envelope in the wet-fixation, more especially in the direction of length. As mentioned above, no envelope, distinct from the general body-substance, can be made out in the living crescents, nor is it obvious, as a rule, in the parasites on Giemsa smears. A point to notice is that the envelope never stands off from the general protoplasm at the point where the flagellum is attached; this indicates that the latter organella is not merely a development from the membrane, but originates

[1] This splitting is never seen in the living parasites, but it is interesting to note that we have observed a somewhat similar splitting of the flagellum (in life) in a "true" flagellate occurring in the rumen (perhaps a Sphæromonas), which possesses a long, thick, curved flagellum.

from the general protoplasm. We may say here that there is not the faintest hint of any groove or depression around the body of a crescent at the point where the flagellum starts.

Not the least remarkable feature of Selenomastix is its cytology. The best stain is undoubtedly iron-hæmatoxylin. Delafield's hæmatoxylin shows just the same minute structure, but it has the drawback that the flagellum is usually very faintly stained and often cannot be made out, and the same remark applies to carmine stains. Giemsa's stain is of considerable use in many respects, but not of much service in bringing out the details of the internal structure of the body, except where the chromatin is in the form of one or two prominent masses. The general protoplasm nearly always stains uniformly and homogeneously, sometimes lighter and sometimes darker, according to the degree of extraction. It never shows either granules of any kind or vacuoles.

There is no properly constituted nucleus, either of the usual karyosomatic type seen in the flagellates, or of any other type with which we are acquainted. Nevertheless, chromatin is undoubtedly present in greater or less quantity, occupying a peculiar but characteristic position. The principal situation of the chromatinic substance is at the periphery of the body. In the condition in which the parasite has apparently the least amount of chromatin, this constitutes a very narrow layer or zone, extending all over the surface of the body and appearing in optical section as a definite border, staining blacker and more intensely than the cytoplasm (figs. 3, 5, 6). More generally, however, this layer shows distinct thickenings, which may take the form either of numerous fine, small granules, appearing as little more than dots (figs. 1, 4, 8); or of few or several larger more conspicuous grains or small masses (figs. 9, 11, 10, 17); or, finally, of a few (usually one or two) quite large, dark-staining masses (figs. 12–16, 26). The granules and masses project inwards, one edge always being at the surface of the body, and we are inclined to consider them as having developed from the basal, peripheral zone or layer. Now and again these dark masses

form thick half-hoops or rings, partially encircling the body (figs. 18, 19). In large individuals they may occur together with conspicuous granules in the peripheral zone (fig. 25), but as a rule in the smaller forms, when a prominent chromatinic mass is present, the peripheral zone appears to contain very little chromatin.

Division.—Whenever the crescents have occurred in numbers we have found division proceeding actively. Division of the parasites always takes place by means of equal binary fission. We have never seen the slightest indication of unequal fission, or of anything in the nature of budding. So far as we have been able to ascertain, binary fission appears to be the only form of multiplication in Selenomastix. The division always takes place in a plane at right angles to the long axis of the body, i. e. it is transverse to it. Division does not stand, apparently, in any definite relation to the size of the parasite; that is to say, not only large individuals divide, but intermediate-sized ones and also quite small forms. Neither does the condition in which the chromatinic substance is present appear to determine fission, for individuals can be found undergoing division in which the chromatin is practically in any of the states described above (cf. figs. given of dividing forms).

In the great majority of cases, though not by any means always, the fission is initiated by the splitting of the flagellum along the greater part of its length. This is shown clearly in figs. 20, 23. There is no question of this appearance being merely a fraying-out of the flagellum into fibrils, such as was referred to above. For one thing, the instances figured (and others observed) are on wet-fixed films, in which the fraying-out is never found. Again, when the flagellum shows a frayed-out appearance, it is either the middle portion or else the free distal end which is split into fibrils of varying thickness; in the true splitting of the flagellum, leading to division, the basal part divides first of all into two daughter-flagella of equal thickness, the proximal, attached ends first separating. In the figures mentioned, the splitting has not

yet proceeded along the entire length, and the two daughter-flagella are still united into one distally. Probably the parent-flagellum splits nearly throughout its length, for the two daughter-flagella are usually approximately equal (figs. 24–28); it is exceptional to find a dividing individual in which there is as great a difference between the length of the separated flagella as in that of fig. 31. We have been considerably exercised in regard to the question whether there is a basal granule in connection with the flagellum. We are rather inclined to think that there may be such a granule, but we cannot say with certainty. There is frequently a definite granule exactly at the point where the flagellum originates (figs. 11, 13, 21 and 22), but owing to the peripheral situation of the chromatinic zone, it is possible, of course, that such granule is a chromatin grain. Nevertheless, in such a case as is shown in fig. 20, where the chromatinic zone is very feebly developed, but where there is a very distinct granule-like thickening at the basal end of each of the daughter-flagella, definite basal granules are certainly suggested.

As regards the division of the chromatinic substance, we have found nothing to indicate that there is any pronounced attempt at equal distribution between the two daughter-individuals. Apparently, the chromatic substance which happens to be in either half, prior to division, goes to that daughter-individual (cf. figs. 24 and 25). However, the most usual condition in which the chromatin occurs in dividing individuals is that of a number of small granules fairly uniformly distributed around the periphery (figs. 27, 28), and therefore there may be really a nearer approach to equalisation than is obvious owing to the absence of a definite nucleus. The last act in the process is the constriction of the general body-substance into two halves; this always takes place exactly in the middle of the long axis.

In some cases fission of the body undoubtedly occurs before the flagellum has split (figs. 32, 30, 29 show different stages in such a process). Whether the daughter-individual which thus lacks a flagellum is able to develop one we cannot say,

but crescents which do not possess a flagellum certainly occur (figs. 36, 37). We are inclined to think that such forms may become ovals, which we have next to consider.

The Ovals.—In their general appearance the ovals resemble the crescents. Their average size also is quite comparable; they are somewhat shorter, but distinctly more bulky. Some of them are seen to be considerably elongated, but these are individuals either about to divide or in the act of dividing. Apart from their shape the essential point of difference from the crescents is that the ovals entirely lack the characteristic flagellum. Nevertheless the ovals are undoubtedly capable of movement, and this fact was brought home to us in a surprising manner. When the fourth goat was examined for the first time an enormous number of active ovals were found, while the crescents were extremely scanty —far fewer in number than on any other occasion. The great majority of the ovals were in motion, the movement being one of progression, in a slightly zig-zag manner, but no sudden reversal of the direction of movement was noticed. At each of the subsequent examinations of the same goat, when the ovals have been relatively fewer and the crescents more numerous, most of the ovals have been quite still (although there were active crescents in the same preparations). Here and there, however, an oval would be seen zig-zagging to and fro slightly and spasmodically, scarcely progressing at all. And this has been the case in most of the other goats examined; nearly all the ovals were motionless. We have not observed any rotation of the ovals on their own axis, such as is commonly seen in the crescents. The remarkable activity of the ovals on the particular occasion referred to soon subsided, and after about a couple of hours they were all still. In the last goat examined, however, many of the ovals, as well as the crescents, were active, and we were able to observe a distinct indication of antero-posterior polarity in their case also. Now and again an oval steadily progressing would come against an obstacle. When this occurred the oval did not move away in the opposite sense, but turned

quite round and went off in another direction, the same end still being in front.

We had some of these active ovals specially stained for us by one of the principal methods in use (de Rossi's) for showing up the flagella of bacteria, but with no result whatever. We have ourselves tried this method and also Pitfield's method, with equally negative results. In short, in none of our preparations, however stained, have we ever seen a flagellum or tuft of flagella in connection with an oval; and we feel convinced that the ovals do not possess flagella of any kind. We are supported in this view by two points: (1) The close agreement in minute structure shown by many of the ovals and crescents, and the fact that the flagellum of the latter is a well-developed structure, readily visible; and (2) the conviction we have gained that the crescents themselves are capable of movement by other means than their flagellum.[1]

While many of the ovals have the same homogeneous appearance in life as the crescents, in some the membrane or envelope stands out distinctly, being separated from the general body-substance by a narrow, clear area (Text-figs. H, L and M). In the majority of the ovals, the envelope appears to be more prominent and more distinct from the body than in the crescents, and this is borne out by the study of stained preparations. Probably it is a firmer, more resistant structure in the ovals.

Structure.—The ovals are rarely, if ever, spherical; the nearest approach to a spherical shape is seen in forms immediately resulting from division (figs. 44, 55, 57), and even in these one diameter is usually greater than the other. On the other hand, they are rarely sufficiently long in proportion to their width, and the two longer sides sufficiently straight and parallel, for them to be regarded as rod-like; here, again, the nearest approach to such an appearance is shown by those forms about to divide (fig. 56). Undoubtedly the oval

[1] It may be added, perhaps, that neither do the crescents show any flagella of the bacterial type when stained by the above-mentioned special methods.

shape is typical of this phase of the parasite. The average size varies from 7–9½ μ in length, by 3½–5 μ in width; individuals with dimensions less than these occur, but larger ones are nearly always in the act of dividing; the individual of fig. 48 is about the largest single oval found.

In the case of the ovals, the minute structure can be made out satisfactorily only in wet-fixed preparations, stained by hæmatoxylin; in Giemsa smears, the ovals—especially the large ones—stain much more intensely than the crescents, and usually appear rather blotchy, the stain being deposited to a greater extent either in, or immediately beneath, the (thicker) envelope. Hence, only two or three examples

TEXT-FIGS. H–M.

Ovals drawn either living or after fixation with osmic acid vapour. H, L and M show the envelope distinctly; in J and K it is not visible.

stained in this manner are figured, for the sake of comparison (figs. 83–87). The ovals show two types of minute structure, which, at first sight, appear quite different; we think, however, that they are connected by intermediate conditions. The first type of structure is practically identical with that of many crescents. There is just the same difference with regard to the distinctness of the envelope. In many individuals it stands off well from the general protoplasm at the two ends of the body (figs, 39, 40, 53); in others, though these are fewer in number, it is not apparent at all (figs. 41, 43, 45, 54). The protoplasm stains in the same uniform manner and shows neither granules nor vacuoles. The chromatinic substance is distributed in the same characteristic manner, constituting usually a zone of

fine granules closely arranged round the periphery (figs. 39–41), or, more rarely, comprising fewer, somewhat more prominent granules (figs. 45, 46, 49). We have not found any ovals with one or two large, deeply staining masses of chromatin such as are shown by some of the crescents. Further, the ovals divide in just the same way, by equal, transverse, binary fission (figs. 49–53). Whatever the crescents are, we think there can be no doubt that these ovals are, at any rate, a very similar type of thing (cf. especially figs. 6, 27 of crescents with figs. 40, 53 of ovals); the only essential point of difference is that the latter have no flagellum.

In the great majority of the ovals which show the second type of minute structure, the envelope projects markedly at both ends of the body (figs. 65–69), and now and again it stands off slightly also at the sides (figs. 64, 66, and fig. 87 on a Giemsa smear). The general protoplasm is usually sharply divided into two distinct zones, a central, lighter-staining region and a peripheral, more deeply staining area, which is usually wider at the two ends. The lighter staining, central area appears very similar to the general cytoplasm of the other ovals, and is, we consider, comparable to that. The darker-staining zone appears practically homogeneous, and does not contain, or is not composed of, the fine intensely staining granules characterising the chromatinic zone of the first type of ovals. The comparative extent of the central pale area and the surrounding darker region varies greatly in different individuals. In some the central area is small and the dark zone thick and broad (figs. 65, 67, 68); in others the paler area is much increased and the peripheral zone reduced to a narrow band (figs. 58, 59). Frequently, with this increase of the paler area, the dark-staining substance persists chiefly in the form of two caps, one at each end of the oval, connected only along the two sides of the oval by an extremely thin peripheral layer (figs. 60, 69). Lastly, in a small proportion of ovals, all the protoplasm appears to consist of the darker-staining substance (figs. 64, 70); these may be either small or fairly large.

We have not found such well-marked indications of division in ovals possessing a large area of dark-staining material as in those of the other type; but we are inclined to think that the same transverse binary fission occurs. A not uncommon feature in ovals of this type is the presence of two definite, intensely staining granules, one at the middle of each of the longer sides of the body (figs. 67, 69–71). Frequently these two granules are connected by a fine line, which is sometimes seen to follow the external contour of the body (fig. 70), when it probably represents a very slight annular constriction across it; but at other times the line can be traced with difficulty through the body (fig. 69). It seems probable that these appearances indicate transverse division, but we do not think it occurs to nearly the same extent in ovals showing this second condition of the internal structure.

We may now consider briefly the question of the connection of these different types of form with each other, and of their association together as different phases of one parasite. In the first place, ovals showing the second type of minute structure can be readily connected with those showing the first condition described, by a series of intermediate stages. All degrees in the thinning out of the darker staining area until it is little more than a narrow peripheral ring (as in fig. 59) can be found; and from such a stage to that shown by the individuals, for instance, of figs. 39 or 40 is a very slight transition. Another marked transition stage is seen in fig. 42, where the narrow peripheral, intensely staining zone is slightly thickened around one end; such a condition is manifestly closely connected with that showing a cap of dark-staining substance at each end (as in figs. 60, 61). It is a little difficult to know what interpretation to assign to this darker-staining part of the protoplasm, as found in the second type of oval. In the ovals with a well-marked, finely granular peripheral layer, or with more conspicuous granules, we consider that this zone comprises the chromatinic material of the cell, just as in the case of the crescents. Are we, then, to regard the more or less homogeneous, darker staining

region, when present, as representing chromatin or some allied substance diffused in an extremely fine condition throughout a relatively large area of the protoplasm?

Secondly, as regards the first type of ovals and the crescents, there are several reasons for concluding that these are only distinct phases of one parasite. There is the fact that, on all occasions save one, we have found the two forms associated, and possibly in the case of the third goat ovals may have been present, but were so scarce in comparison with the enormous number of crescents that we did not notice them. Important points of agreement between the two types as regards appearance, one manner of movement, structure, the occurrence of crescents without a flagellum, and so on, have been already dealt with. Lastly, in the case of some individuals, it is purely a matter of choice whether to regard them as bean-like crescents, or as bean-like ovals; thus the form drawn in fig. 38 is readily derivable, one may reasonably suppose, from an aflagellate crescent such as that of fig. 36, while on the other hand, between the parasite of fig. 46 and the oval of fig. 45 there is equally little difference.

Concluding, then, that the above-described different forms all belong to one parasite, it still remains a matter of uncertainty what is the order of transition between them, respectively, and how the different phases should be combined into one life-cycle; it appears very probable that the crescents can give rise to ovals; but we have no indications as to whether the ovals become crescents. Further, we are inclined to the view that the second type of ovals pass into the first type, rather than vice versâ.

The Nature and Affinities of Selenomastix ruminantium.—It will be apparent from the foregoing account that this new parasite does not fall readily into any of the known groups of organisms included under the designation Protista; in many respects it is an altogether new type of organism. On first seeing the living, active crescents, with their conspicuous, long flagellum, we naturally thought we had to deal with a new member of the flagellates, as, indeed,

was Certes' opinion originally. From a further study of Selenomastix, however, we feel at present very doubtful whether it is a true flagellate. Supposing for the moment that it is, the question of the orientation of the body is a very important one, because this determines, of course, the nature of the division. We have not the slightest indication that the middle of the concave side—approximately the point of insertion of the flagellum—represents the anterior end; on the contrary, such evidence of antero-posterior polarity as we have obtained points to this being in the direction of the longer axis of the body, both in the crescents and the ovals. Hence we must regard the division as transverse. Apart from the entire order of the Dinoflagellates, there are scarcely any Flagellates in which division is transverse. We can find no hint whatever of Dinoflagellate characters in Selenomastix. In a crescent which is not commencing to divide, there is neither a second flagellum nor any sign of an annular, transverse groove. It is equally difficult to see any indication of relationship among the Euflagellates. For one thing, the peculiar scattered or diffuse condition of the chromatinic substance is very different from the definite nucleus which is typical of flagellates. Another point which in our view weighs very much against the flagellate affinity of this new creature is the conviction we have that it is capable (either in the crescent or the oval phase) of moving by means of its body alone, somehow, independently of the flagellum, when this is present. We have next to search, therefore, among the vast assemblage of organisms collectively known as bacteria for a clue to the relationships of Selenomastix.[1]

[1] So far as the ovals alone were concerned, we did not overlook their possible connection with some of the Saccharomycetes, such as Schizosaccharomyces. Thanks to the kindness of Dr. Harden, of the Lister Institute, we have been able to compare the ovals with organisms of this group, and it was at once apparent that with them they have nothing whatever to do. Further, the ovals do not resemble in any way another yeast-like type of organism, namely Blastocystis, which has been lately described.

We have considered it useful to discuss the possible relationship of Selenomastix to the flagellates, because not only does our parasite differ in many respects from any bacterial protist of which we have knowledge, but it also appears to incline more to the Protozoa in one or two important features. In the first place, the host of ordinary bacteria may be at once dismissed from consideration. Dr. Ledingham has kindly looked at the parasites and entirely agrees with us in this opinion; moreover, as above mentioned, cultures made on various media were quite unsuccessful. The most striking feature of Selenomastix, from a bacterial point of view, namely, the presence of a large flagellum, easily visible in life and by ordinary staining methods, is only met with, so far as we are aware, in the case of one or two very large spirillar forms and among certain "Sulphur-Bacteria" (Rhabdomonas, Ophidomonas), of which a good account has been given by Bütschli ('Arch. Protistenk.,' vol. i, 1902, p. 41). The spirillar forms and Ophidomonas have a flagellum at each end, Rhabdomonas a single, terminal one. In these forms, too, the flagellum shows a tendency to split up, in a somewhat similar manner, into fibrils of varying thickness. These forms have also a well-marked envelope (periplast), which stand off well from the body in many cases; that of Rhabdomonas is spirally striated, a point which we have never seen in Selenomastix.

It is possible that the origin of Selenomastix is to be sought amongst this type of organism; we have had it tested for the presence of sulphur, however, with entirely negative results. Moreover, Selenomastix certainly appears very far removed from the spirillar type as generally recognised. Taking first the points of agreement, there is, of course, the transverse division and the absence of a definite, constituted nucleus. Another feature which is somewhat against the Protozoan character of our parasite is the peculiar homogeneous appearance of the protoplasm; the ordinary true flagellates, for instance, which occur in the rumen, look very

different, with their granular cytoplasm. In this respect Selenomastix agrees with many bacteria, though it so happens that spirilla, especially the larger ones, are often distinctly granular and sometimes exhibit a chambered structure, of which there is no sign in this new form.

The principal differences from the spirillar type are as follows: There is no true spiral form of the body, or indication of spiral movement. In some individuals, however, there is a hint of a twisting of the axis towards one end, which may or may not represent a permanent condition. There are no terminal flagella, but a single more or less median one. There is distinct evidence of antero-posterior orientation. On the other hand, we have never observed the reversal of direction characteristic of spirilla. Another important distinction is that both the crescents and the ovals can move by means of the body alone, resembling a Spirochæte, with which, however, Selenomastix has assuredly nothing else in common. Again, individuals certainly vary in width as well as in length; broadly speaking, the larger individuals are both longer and wider than the smaller ones; this is apparent from our plates. Last, but not least, no spirilla of any kind hitherto described, so far as we know, have any phase connected with them comparable to the ovals of Selenomastix.

With regard to the vexed question of plasmolysis, we are inclined to think that this occurs, at any rate in the ovals. In living preparations which have been made for some time (whether diluted with a drop of normal saline solution or not), a small proportion of the ovals show a vacuole, or space-like appearance near one or both ends; this is most probably due to the shrinkage of the protoplasm away from the envelope. When the parasites are placed in 5 per cent. or in 10 per cent. salt solution, a somewhat larger proportion of the ovals show this shrinkage appearance, and it is evident, also, here and there in a few crescents. Many of the ovals, however, and the great majority of the crescents—chiefly those, we think, in which, if stained, the envelope would not stand off

in a marked manner—do not appear to be altered at all. They do not swell up, burst, or undergo any other obvious change. An interesting fact, moreover, which we noticed was that, when such preparations were looked at again the next morning, there did not appear to be as many ovals showing the contracted protoplasm as there were soon after the preparations were made. It seemed to us as if the protoplasm must have expanded again in some individuals, which could not, therefore, have been dead.

Of one thing we are sure, namely, that Selenomastix does not undergo what the Germans term "Präparationsplasmolyse." This is evident from our figures, which give a fair assortment of the various appearances seen in the stained preparations. Lest it might be thought that the condition in which there are one or two deeply staining masses (regarded by us as chromatinic) in the cell, represents such an artifact, we may point out, first, that there is no larger proportion of such individuals on "dried" Giemsa smears than occurs in properly "wet-fixed" films, made as soon after removal from the rumen as possible; secondly, that every transition can be traced in the development of this phase where the chromatinic substance is compacted into few masses, through conditions where there are a varying number of smaller, but quite prominent granules; and finally, if it were an artifact, the ovals with the first type of minute structure, closely comparable to that of the crescents, might be expected also to show it, which is never the case.

We have now, we think, considered exhaustively the possible directions in which to look for the origin and affinities of this remarkable parasite, so far as we have been able to do so from the facts we have learnt with regard to it up to the present. To sum up, it appears to be entirely unconnected with the Dinoflagellates; it may possibly be derived from some large spirillum, or from an Ophidomonas- or Rhabdomonas-like form, although we are very doubtful upon the point. For our own part, we are inclined to hazard the suggestion that if there is such a thing as a Pro-Protozoan or

Pro-Flagellate, Selenomastix ruminantium (Certes) represents such a Protist, because of the fact that it exhibits certain characters which are common to the flagellate Protozoa, but which are rarely or never possessed by bacteria.

The Lister Institute,
July, 1913.

Summary.

(1) This paper describes a new type of parasitic Protist, to which we have given the name Selenomastix ruminantium (Certes). Its habitat is the rumen of Ruminants, especially that of the goat.

(2) The organism occurs in two chief forms—crescents and ovals. The crescents present a homogeneous, non-granular appearance, and possess a definite envelope; a single, large flagellum, conspicuous in life, arises from about the middle of the concavity of the crescent. The method of movement is variable; while the movement is sometimes effected by the flagellum, in other cases, perhaps more usually, it is produced by the body alone. In forward progression distinct antero-posterior polarity can be recognised. There is no properly constituted nucleus, the chromatin being present in the form of a peripheral layer, in which granules of varying size may occur, or there may be one or two large masses projecting into the cytoplasm. Division is by equal binary fission, transverse to the long axis.

(3) The ovals resemble the crescents in general, but they never possess a flagellum, although capable of active movement. They show two types of minute structure: (A) ovals in which the chromatinic substance occurs as a narrow, peripheral layer, with or without granules in it. This arrangement agrees closely with that found in the crescents. (B) Ovals in the protoplasm of which two zones can be distinguished, a central, lighter-staining zone, comparable to the cytoplasm of (A) and of the crescents, and a peripheral, darker area of variable extent. This latter may be chromatinic in nature.

(4) We suggest that the second type of oval gives rise to the first type, and also that the crescent may pass into the first type of oval by the loss of the flagellum. We have no indication whether the crescents may be developed from the ovals or not.

(5) Apparently the only Flagellates from which this organism could be derived are the Dinoflagellates, and, apart from the transverse division, there is no indication of any affinity with this group. Further, the nature of the "nucleus" and the capacity of moving by the body alone make it very doubtful if this parasite is a true protozoan.

(6) Selenomastix ruminantium differs in important respects from any known bacteria. It has no affinities with Schizo-saccharomycetes, with Blastocystis, nor with the Spirochætes. In certain characters it shows a resemblance to one or two large Spirillar forms, or to certain members of the Sulphur-Bacteria (e.g. Ophidomonas), but while its derivation is possibly to be sought in this direction, it is, nevertheless, very far removed from such forms. We may have in Selenomastix an example of a Pro-flagellate.

EXPLANATION OF PLATES 29 AND 30.

Illustrating the paper by Dr. H. M. Woodcock and Mr. G. Lapage, "On a Remarkable New Type of Protistan Parasite."

[All the figures are magnified 2000 times linear. We are indebted to Miss Rhodes for kindly drawing a few of them.]

Figs. 1–38.—All the figures are of crescents, and are from "wet-fixed" films, stained by iron-hæmatoxylin.

Figs. 1–19.—Single individuals, of various size, showing different conditions of the envelope and of the chromatinic substance.

Figs. 20–26.—Dividing individuals possessing two flagella, but in which the body does not yet show indications of fission. In figs. 20 and 23 the actual splitting of the flagellum is shown.

Figs. 27, 28, and 31.—Later stages of fission, in which the body is also dividing.

Figs. 29, 30, and 32.—Individuals in which the body is dividing, but of which the flagellum has remained single. One of the daughter-individuals will be aflagellate.

Fig. 33.—A small individual in the very last stage of division.

Figs. 34 and 35.—Small pyriform individuals, probably immediately resulting from fission.

Figs. 36 and 37.—Aflagellate crescents.

Fig. 38.—Bean-like crescent, which may be transitional to an oval.

Figs. 39–71 are of ovals, from "wet-fixed" films, stained by iron-hæmatoxylin.

Figs. 39–57.—Ovals with the first type of minute structure.

Figs. 39–48, 54.—Single individuals of various size.

Figs. 49–53, 55 and 57.—Individuals showing different stages of fission.

Figs. 58, 60–62, 64–68.—Single individuals showing the second type of minute structure.

Figs. 59, 61.—Individuals transitional between ovals of the first and second type.

Fig. 63.—Small dividing individual.

Figs. 69–71.—Ovals showing two definite granules at opposite sides, connected by a line or ring (see text). The individual of fig. 71 is apparently beginning to divide.

Figs. 72–82 are of crescents stained by Giemsa.

Figs. 72 and 73.—Single individuals; flagellum normal.

Fig. 74.—Dividing individual; flagella normal.

Figs. 75–77.—Individuals showing artificial fraying-out of the flagellum.

Fig. 78.—Smallest crescent found on a Giemsa smear.

Fig. 79.—Dividing individual, the upper flagellum of which shows indication of fraying-out.

Figs. 80–82.—Aflagellate crescents, showing one or two conspicuous chromatinic masses.

Figs. 83–87.—Ovals stained by Giemsa. In fig. 87 the envelope stands off markedly from the body and shows an annular line (cf. figs. 70 and 71).